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Award Number: DAMD17-01-1-0015

TITLE: Role of IKKs and Transcription Factor NF-kB in Prostate
Tumorigenesis

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REPORT DATE: May 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20031028 264

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE May 2003	3. REPORT TYPE AND DATES COVERED Annual (1 May 02 - 30 Apr 03)	
4. TITLE AND SUBTITLE Role of IKKs and Transcription Factor NF- κ B in Prostate Tumorigenesis			5. FUNDING NUMBERS DAMD17-01-1-0015	
6. AUTHOR(S) Irina V. Budunova, M.D., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AMC Cancer Research Center Lakewood, Colorado 80214 E-Mail: budunovai@amc.org			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) The goal of this project is to understand the role of IKK kinases and NF- κ B "survival signaling" pathway in tumorigenesis in prostate. In our previous studies we found that NF- κ B was constitutively activated in human androgen-independent prostate carcinoma (PC) cell lines. During the current funding period we found that one of the IKKs, IKK ϵ was only expressed in androgen-independent PC cells with high level of constitutively active NF- κ B. We obtained experimental evidence that IKK ϵ could be involved in the NF- κ B induction in PC cells through a positive feedback loop. We also found that the conditioned medium from PC cells induced NF- κ B in cells with low level of NF- κ B activity through secreted proteinaceous factor. These data strongly suggest that NF- κ B activity in androgen-independent PC cells may be mediated through an autocrine/paracrine loops. We have generated 175 clones of PC cells transfected with d.n. mutants of different IKKs. We have assessed the effect of d.n. IKK mutants on NF- κ B in clones with the highest level of d.n. IKK expression. The IKK α did not significantly affect NF- κ B function in PC cells; IKK ϵ d.n. mutant moderately affected NF- κ B activation by selective NF- κ B inducers; IKK β and IKK γ mutants significantly blocked transcriptional activity of NF- κ B in PC cells.				
14. SUBJECT TERMS Prostate carcinoma, NF- κ B, IKK kinase, apoptosis, tumorigenesis				15. NUMBER OF PAGES 20
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Introduction

The overall goal of this project is to understand the role of up-stream I κ B kinases (IKK) and NF- κ B "survival signaling" pathway in tumorigenesis in prostate. During first year we found that NF- κ B was constitutively activated in human androgen-independent PC cell lines due to the constitutive activation of IKK α/β kinases. During the current funding period we continued to study the mechanisms of constitutive NF- κ B activation in PC cells, and also as was planned, focused on the stable transfection of PC cells with d.n. mutants of IKK α , IKK β , and IKK ϵ . We have added to the panel of IKKs under study IKK γ (also called NEMO), an important component of the IKK complex essential for NF-kappaB activation (Yamaoka et al., 1998, Israel, 2000). Our results indicate that there are positive feedback loops as well as autocrine/paracrine loops involved in constitutive activation of NF- κ B in PC cells. We have obtained the experimental evidence that IKK ϵ is strongly involved in constitutive activation of NF- κ B in androgen-independent PC cells. Our studies have resulted in publication of one peer-reviewed article. One more manuscript has been submitted. The following describes progress made in this year.

Body

As was proposed we focused during second year on the tasks 6-8 pertinent to the part of the Specific Aim 1 of proposal as well as to the part of Specific Aim 2. We have also conducted experiments to elucidate the additional mechanisms of constitutive NF- κ B activation in PC cells.

Our mechanistic studies were more profoundly focused on the inducible IKK ϵ (also called IKKi) - the only IKK whose activity is regulated by its expression Shimada et al., 1999, Peters et al., 2000). We found that IKK ϵ was strongly expressed only in androgen-independent PC cells (PC3 and DU145) with high level of constitutively active NF-kappaB but not in androgen-dependent PC cell lines and primary prostate epithelial cells (Yao et al., 2002). Immunostaining also revealed that IKKi was expressed in human prostate carcinomas. Treatment of PC cells with NF-kappaB inducers such as IL-1 and TNF- α resulted in a rapid induction of IKK ϵ (Yao et al., 2002). Transient transfection of different PC cell lines with IKK ϵ w.t. resulted in activation of κ B-Luciferase reporter, whereas IKK ϵ dominant negative (d.n.) mutant K38A suppressed basal NF- κ B activity in PC cells. These data provide experimental evidence that IKK ϵ could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop.

We also found that the conditioned medium from PC3 cells with activated NF- κ B, induced NF- κ B in LNCaP cells with low level of NF- κ B activity. Conditioned medium taken from PC3 cells induced I κ B α phosphorylation as well as κ B DNA-binding in LNCaP cells (Gasparian et al., 2002). It also increased the expression of endogenous κ B-responsive genes such as I κ B- α and IL6. Boiling and multiple freezing/thawing of PC3 conditioned media eliminated its effect on κ B DNA-binding. This suggests that secreted NF- κ B inducer is a proteinaceous factor. All together these data allowed us to advance

the hypothesis that NF- κ B activity in androgen-independent PC cells may be mediated through an autocrine/paracrine loop.

As planned, during the current funding period we focused on the stable transfections of prostate carcinoma cell lines with dominant negative (d.n.) constructs of upstream kinases IKK α , IKK β , and IKK ϵ . We have also added to the panel of IKKs under study IKK γ (also called NEMO), an important component of the IKK complex essential for NF- κ B activation. We have generated more than 95 clones of DU145 and more than 80 clones of PC3 cells transfected with d.n. mutants of different IKKs. We have selected positive clones with the highest level of transgene expression and assessed the effect of d.n. IKK mutants on NF- κ B function in PC cells using electromobility shift assay (EMSA), Northern blotting for endogenous κ B-responsive gene expression, and transient transfection with κ B.Luciferase reporters. We also studied the effect of IKK d.n. mutant stable expression on I κ B- α degradation induced by different κ B inducers such as TNF- α , IL-1, TPA and LPS. The obtained results suggest that IKK α d.n. mutant did not strongly affect NF- κ B function in PC cells. We found that in PC clones transfected with IKK ϵ d.n. mutant constitutive expression of endogenous κ B-responsive genes I κ B- α and IL6 was moderately decreased (Yao et al., 2002). The IKK ϵ d.n. mutant also moderately affected NF- κ B activation by selective NF- κ B inducers such as IL-1 and TPA (Yao et al., 2002). On the other hand, IKK ϵ d.n. mutant did not affect activation of NF- κ B-binding by TNF- α even though the degradation of I κ B- α in IKK ϵ d.n.-transfected cells was inhibited in comparison to parental and empty vector-transfected PC3 cells. Our experiments have revealed that the expression of endogenous κ B-responsive genes was dramatically inhibited in PC3 clones stably transfected with d.n. IKK γ and d.n. IKK β mutants. The effect of IKK γ d.n. and IKK β d.n. mutants on κ B DNA binding is currently under study.

Key Research Accomplishments

- ❖ We found that IKK ϵ is highly expressed in human androgen-independent PC cell lines and human prostate carcinomas.
- ❖ Our experimental data clearly suggest that IKK ϵ is involved in the NF- κ B activation in PC cells through a positive feedback loop.
- ❖ PC cells with high level of NF- κ B activity secrete a proteinaceous factor/factors that can induce NF- κ B in cells with low level of NF- κ B activity.
- ❖ We have generated more than 95 clones of DU145 and more than 80 clones of PC3 cells transfected with d.n. mutants of IKK α , IKK β , IKK ϵ , and IKK γ .
- ❖ NF κ B function was dramatically inhibited in PC cells stably transfected with d.n. IKK γ and d.n. IKK β mutants. D.n. IKK ϵ mutant moderately affected NF- κ B activation by selective NF- κ B inducers.

Reportable outcomes

Manuscripts

1. Gasparian, A.V., Yao, Y.J., Lu, J., Slaga T.J., and Budunova, I.V. Selenium compounds inhibit I κ B kinase and transcriptional factor NF- κ B in prostate cells. *Molecular Cancer Therapeutics, Mol Cancer Ther.* 1(12):1079-87, 2002.
2. Gasparian, A. V., Yao, Y. J., and Budunova, I. V. High sensitivity of prostate carcinoma cell lines to NF- κ B induction. Submitted.

Abstracts presented at national meetings

1. Gasparian A.V., Yao Y.J., Lü J., Slaga T.J. and Budunova I.V. Selenium compounds inhibit I κ B kinase and transcriptional factor NF- κ B in prostate cancer cells. Keystone Symposium: NF- κ B: bench to bedside. February 25-March 3, 2002, Keystone, Colorado, p. 60.
2. Gasparian, A. V., Yemelyanov A.Yu., Yao, Y. J., Kowalczyk, D., and Budunova, I. V. Prostate carcinoma cells secrete a soluble factor(s) responsible for NF- κ B activation. *Proceedings of AACR.* 43: 364, 2002.
3. Yao, Y. J., Kowalczyk, D., and Budunova, I. V. Role of IKK ϵ in NF- κ B activation in prostate carcinoma cells. *Proceedings of AACR.* 43: 549, 2002.

Seminar presentations by P.I.

1. Selenium compounds inhibit I κ B kinase and transcriptional factor NF- κ B in prostate cancer cells. Basic Science Conference, Division of Medical Oncology, University of Colorado Health Sciences Center, Denver, CO. February 2002.
2. Constitutive activation of NF- κ B in prostate carcinoma cells: possible role of feedback loops. Prostate Cancer Research Group meeting, University of Colorado Health Sciences Center, Denver, CO, July, 2002.

Conclusions

Our data suggest that constitutive NF- κ B activity in androgen-independent PC cells may be mediated through an autocrine/paracrine loops and through feedback loop involving IKK ϵ . Our results also suggest that IKK γ and IKK β play more important role in NF- κ B transcriptional function in prostate cells.

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1. Gasparian, A.V., Yao, Y.J, Lu, J., Slaga T.J, and Budunova, I.V. Selenium compounds inhibit Ikb kinase and transcriptional factor NF-kB in prostate cells. Molecular Cancer Therapeutics, Mol Cancer Ther. 1(12):1079-87, 2002.
2. Gasparian, A. V., Yemelyanov A.Yu., Yao, Y. J., Kowalczyk, D., and Budunova, I. V. Prostate carcinoma cells secrete a soluble factor(s) responsible for NF-kB activation. Proceedings of AACR. 43: 364, 2002.
3. Yao, Y. J., Kowalczyk, D., and Budunova, I. V. Role of IKKe in NF-kappaB activation in prostate carcinoma cells. Proceedings of AACR. 43: 549, 2002.

Selenium Compounds Inhibit I κ B Kinase (IKK) and Nuclear Factor- κ B (NF- κ B) in Prostate Cancer Cells¹

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Abstract

Selenium compounds are potential chemopreventive agents for prostate cancer. There are several proposed mechanisms for their anticancer effect, including enhanced apoptosis of transformed cells. Because the transcription factor nuclear factor- κ B (NF- κ B) is often constitutively activated in tumors and is a key antiapoptotic factor in mammalian cells, we tested whether selenium inhibited NF- κ B activity in prostate cancer cells. In our work, we used sodium selenite and a novel synthetic compound, methylseleninic acid (MSeA), that served as a precursor of the putative active monomethyl metabolite methylselenol. We found that both selenium forms inhibited cell growth and induced apoptosis in DU145 and JCA1 prostate carcinoma cells. Sodium selenite and MSeA, at the concentrations that induced apoptosis, inhibited NF- κ B DNA binding induced by tumor necrosis factor- α and lipopolysaccharide in DU145 and JCA1 prostate cells. Both compounds also inhibited κ B-Luciferase reporter activity in prostate cells. A key to NF- κ B regulation is the inhibitory κ B (I κ B) proteins that in response to diverse stimuli are rapidly phosphorylated by I κ B kinase complex, ubiquitinated, and undergo degradation, releasing NF- κ B factor. We showed that sodium selenite and MSeA inhibited I κ B kinase activation and I κ B- α phosphorylation and degradation induced by TNF- α and lipopolysaccharide in prostate cells. NF- κ B blockage by I κ B- α d.n. mutant resulted in the sensitization of prostate carcinoma cells to apoptosis induced by selenium compounds. These results suggest that selenium may target the NF- κ B activation pathway to exert, at least in part, its cancer chemopreventive effect in prostate.

Introduction

Prostate cancer is the second leading cause of cancer death in men in the United States. Despite significant improvement

of hormonal, chemical, and radiation therapies, there is no cure for locally advanced or metastatic prostate cancer. Thus, studies to develop strategies for prostate cancer prevention continue to be essential. During the past two decades, selenium emerged as a factor with the most consistent anticancer effect among a number of micronutrients tested in animal experiments and clinical trials (1–7). Significantly, selenium appeared to be one of the most promising agents for prostate cancer prevention. The most convincing data have been obtained by Clark *et al.* (3) in a double-blind, placebo-controlled trial involving >1000 patients. Participants treated with a supranutritional dose of selenium in the form of selenized brewer's yeast for 4.5 years (200 μ g of selenium daily, with the average daily intake of selenium in the United States \sim 100 μ g) had substantial reductions in the incidence of prostate cancer, as well as total cancer incidence and mortality (3, 4). The follow-up for the cohort used in Clark's trial further indicated that selenium treatment decreased prostate cancer risk (8).

Selenium is an essential trace element nutrient and is a normal component of diets. The nutritional essentiality of selenium is linked to the functional activities of several enzymes and proteins that contain selenium, including glutathione peroxidases, thioredoxin reductases, and others (2, 9, 10). The chemopreventive activity of selenium is determined by its various covalent forms and metabolites. Over the past 15 years, several groups have focused on the identification of the active metabolite(s) that is critical in selenium cancer chemoprevention and on the search of less toxic forms of selenium that would retain its chemopreventive activity. Selenite is metabolized *in vivo* to a key proximal metabolite hydrogen selenide, which in turn undergoes sequential methylation to methylselenol and other methyl-selenium metabolites (1). Because methylation of hydrogen selenide produces forms of selenium that are not genotoxic (11), stable methylated selenium compounds are currently viewed as precursors or "pro-drugs" to release methylselenol and hold a high potential for cancer chemoprevention use. MSeA⁴ is one of such synthetic compounds that effectively inhibited tumor cell growth *in vitro* (12) and possessed strong antitumor activity in animal experiments (13).

Although several mechanisms including antioxidant protection (via glutathione peroxidases), altered carcinogen metabolism, enhanced immune surveillance, and inhibition of neoangiogenesis have been proposed to account for the anticancer effect of selenium (9, 11), induction of apoptosis

Received 12/26/01; revised 8/12/02; accepted 8/19/02.

¹ Supported by Department of Defense Prostate Cancer Research Program DAMD17-01-1-0015.

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⁴ The abbreviations used are: MSeA, methylseleninic acid; I κ B, inhibitor of NF- κ B; IKK, I κ B kinase; LPS, lipopolysaccharide; NF- κ B, nuclear factor- κ B; PARP, poly(ADP-ribose) polymerase; GFP, green fluorescent protein; TNF, tumor necrosis factor; EMSA, electrophoretic mobility shift assay; EMSA, electrophoretic mobility supershift assay; FL, Firefly luciferase; RL, Renilla luciferase.

of tumor cells by selenium may be of special significance in the chemoprevention of prostate cancer, which is known for very low proliferative activity (14). Because the transcription factor NF- κ B is a key antiapoptotic factor in mammalian cells, we hypothesized that suppression of the NF- κ B activation pathway may be related to selenium chemopreventive activity in prostate.

The active NF- κ B complex is a homo- or heterodimer composed of proteins from the NF- κ B/Rel family. In non-stimulated cells, NF- κ B resides in the cytoplasm in a complex with the inhibitor protein, collectively called I κ B (15, 16). Most agents that activate NF- κ B use a common pathway based on the phosphorylation of the two NH₂-terminal serines in I κ B molecules I κ B- α , I κ B- β , and I κ B- ϵ with subsequent ubiquitination and degradation of I κ B proteins by the 26S proteasome (17–21). Degradation of I κ Bs results in nuclear translocation of released NF- κ B dimers (p65/p50) and activation of target genes. Signal-induced phosphorylation of I κ B is executed by the recently identified IKK complex containing IKK α and IKK β and regulatory proteins (22).

Although selenium has a strong potential as chemopreventive agent for prostate, the mechanisms underlying its diverse biological effects have been studied mostly in other cancer cell models but not in prostate cells. To test our hypothesis that suppression of the NF- κ B activation pathway may be involved in selenium chemopreventive activity in prostate, we studied the effect of two selenium compounds, selenite and MSeA, on NF- κ B activity and upstream IKK kinases in prostate carcinoma cell lines in parallel with the effect of those compounds on prostate cell growth and apoptosis.

Materials and Methods

Cell Cultures and Treatments. In our work, we used two androgen-resistant prostate carcinoma cell lines, DU145 and JCA1. DU145 prostate cancer cells were purchased from American Type Culture Collection (Rockville, MD). JCA1 prostate cancer cells, originally established by Muraki *et al.* (23), were obtained from Dr. O. Rokhlin (University of Iowa, Iowa City, IA). Both cell lines were cultured in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 10% FBS (HyClone, Logan, UT), 10 mM HEPES, 1 mM sodium pyruvate, 0.01 mM 2-mercaptoethanol, 2 mM L-glutamine, and antibiotics as described elsewhere (24). Cells were treated with LPS (from *Escherichia coli* 026:B6; Sigma Chemical Co, St. Louis, MO), TNF- α (R&D Systems, Minneapolis, MN), sodium selenite pentahydrate (J. T. Baker, Inc., Phillipsburg, NJ), or MSeA (a generous gift from Dr. H. Ganther, University of Wisconsin at Madison, Madison, WI).

Effect of Selenium Compounds on Cell Accumulation and Apoptosis in Prostate Cell Cultures. To study the effect of selenium on cell accumulation, DU145 and JCA1 cells were plated on 35-mm dishes and cultured to 50% confluence. They were treated with MSeA or selenite at a concentration of 1–5 μ M without medium change for 24 h. The number of cells in three dishes/treatment was determined.

We used PARP proteolysis and DNA fragmentation assays to determine apoptosis 24 h after the beginning of selenium

treatment. Adherent cells and detached floating cells were combined for whole-cell protein extract preparations. PARP cleavage was estimated by Western blot analysis with PARP antibodies (PharMingen, San Diego, CA).

For DNA fragmentation assay, DNA was isolated from adherent cells and detached floating cells as described previously (12). Briefly, cells were lysed in a buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM EDTA, 0.5% SDS, 0.5 mg/ml proteinase K, and digested at 50°C for 3 h. The lysate was extracted twice with phenol-chloroform. Nucleic acids were precipitated with isopropanol in the presence of 0.2 M NaCl. The pellet was resuspended in 30 μ l of 10 mM Tris-HCl, 1 mM EDTA (pH 7.5), treated with RNase, and loaded onto 1.5% agarose gel containing 0.1 μ g/ml ethidium bromide for electrophoresis. Gels were photographed using UV illumination.

NF- κ B Blockage by Adenovirus Infection. Prostate cells were seeded on 35-mm dishes and at 50% confluence were infected with type 5 recombinant adenovirus (AdV) construct AdV-d.n.I κ B α encoding GFP and mutant human I κ B α protein with substitution of serines 32 and 36 to alanines (32A36A) or adenovirus encoding only GFP (AdV-control). AdV-d.n.I κ B α virus with deletions of E1 and E3 was generated using the AdEasy1 system. The AdEasy1 system was a generous gift of Dr. T.-C. He, (The Howard Hughes Medical Institute, Baltimore, MD). Mutations of I κ B α were constructed by site-directed mutagenesis with the Bio-Rad Muta-Gene Phagemid In Vitro Mutagenesis system (Bio-Rad Laboratories, Hercules, CA). I κ B α mutant has an NH₂-terminal tag (ADRRIPGTAEENLQK) derived from the equine infectious anemia virus tat protein. Control E1/E3-deleted AdV 5 with GFP (AdV-control) was purchased from Quantum Biotechnologies (Montreal, Canada). Adenoviruses were purified by CsCl gradient centrifugation. Cells were infected with adenoviruses (10^9 viral particles/dish) in 700 μ l of medium with 0.5% serum overnight. Twenty-four h after infection, cells were treated with selenium compounds for 24 h.

Preparation of Nuclear and Cytosol Protein Extracts. Cells ($20\text{--}25 \times 10^6$) cells were used for each time point. Cells were washed and harvested in cold PBS. Cell pellets were resuspended in homogenization buffer, and nuclear and cytosolic protein extracts were prepared as described previously (25).

EMSA. The binding reaction for EMSA contained 10 mM HEPES (pH 7.5), 80 mM KCl, 1 mM EDTA, 1 mM EGTA, 6% glycerol, 0.5 μ g of poly(deoxyinosinic-deoxycytidylic acid), 0.5 μ g of sonicated salmon sperm DNA, γ -³²P-labeled ($2\text{--}3 \times 10^5$ cpm) double-stranded κ B-consensus oligonucleotide (Promega Corp., Madison, WI), and 5–10 μ g of the nuclear extract proteins. DNA-binding reaction was performed at room temperature for 30–45 min in a final volume of 20 μ l. To determine the composition of NF- κ B complexes, 1.5 μ l of antibodies against p65 (sc-109X) or p50 (sc-114X) were added 30 min after the beginning of reaction, and incubation was continued for additional 30–45 min. Both antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). DNA-protein complexes were analyzed on 6% DNA retardation gels (Novex, Carlsbad, CA). Dried gels were subjected to autoradiography.

Transient Transfection and Measurement of Luciferase Activity. JCA1 prostate cells were plated on 35-mm dishes and at 50% confluency were cotransfected by Tfx-50 reagent (Promega Corp.) with $3 \times \kappa\text{B}$ -Luciferase reporter-FL under promoter with three copies of conventional κB site (Clontech Laboratories, Inc., Palo Alto, CA) and pRL-null construct-RL under minimal promoter (Promega Corp.). Tfx-50 reagent (2.25 $\mu\text{l}/\mu\text{g}$ of plasmid DNA) and the plasmid DNAs (both in doses of 2 $\mu\text{g}/\text{dish}$) were added to the dishes in antibiotic-free, serum-free medium. Twenty-four h after transfection, JCA1 cells were pretreated with selenium compounds for 30 min and treated with TNF- α for additional 6 h. Cells were harvested in the lysis buffer, and the luciferase activity was measured by dual luciferase assay (Promega Corp.) as recommended by the manufacturer. FL activity was normalized against RL activity to equalize for transfection efficacy.

Western Blot Analysis. Nuclear or cytosol proteins were resolved by electrophoresis on 10–12.5% SDS-PAGE, depending on the size of the target proteins, and transferred to Immobilon-P membrane (Millipore Corp., Bedford, MA). Polyclonal anti-p65 (sc-372) and anti-I $\kappa\text{B}\alpha$ (sc-371) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-Ser32 I $\kappa\text{B}\alpha$ antibody was from Cell Signaling Technology, Inc. (Beverly, MA). Monoclonal PARP antibody that recognizes both PARP and cleaved PARP was purchased from PharMingen. Membranes were blocked with 5% nonfat milk in TBST buffer [10 mM Tris HCl (pH 7.5), 150 mM NaCl, 0.05% Tween 20] and incubated with primary antibodies for 1.5 h at room temperature. Anti-phospho-Ser32 I $\kappa\text{B}\alpha$ antibody and PARP antibody required 6 h incubation at 34°C. Peroxidase-conjugated antirabbit or anti-mouse IgG (Sigma Chemical Co.) was used as a secondary antibody. ECL Western blotting detection kit (Amersham Pharmacia Biotech, Uppsala, Sweden) was used for protein detection. The membranes were also stained with Ponceau Red to verify that equal amounts of proteins were loaded and transferred. Quantitative analysis of Western blots was done by One-Scan gel and blot analysis software, Scanalytics, Inc. (Fairfax, VA).

In Vitro IKK Activity Assay. Cells were lysed in TNT buffer [20 mM Tris HCl (pH 7.5), 200 mM NaCl, 1% Triton X-100] with protease inhibitors (25). Immunoprecipitation of 450 μg of total protein was performed with 1 μl of rabbit IKK α (#1997) and IKK β (#4137) antisera (a kind gift of Dr. N. R. Rice, National Cancer Institute, Frederick, MD) in 3 ml of TNT buffer. Two h later, 20 μl of protein A-Sepharose 4B (Sigma Chemical Co.) in TNT buffer were added to each sample and incubated with gentle rotation overnight. Immunoprecipitate was washed three times in TNT buffer with protease inhibitors and two times with kinase buffer without protease inhibitors. Kinase reaction was performed in kinase buffer [20 mM HEPES (pH 7.4), 2 mM MgCl_2 , and 2 mM MnCl_2], containing 2 μCi of [γ - ^{32}P]ATP and I $\kappa\text{B}\alpha$ peptide (1–54) that has only Ser-32 and Ser-36 sites of phosphorylation (Boston Biologicals, Inc., Boston, MA) as a substrate for 30 min at 30°C. Then $2 \times$ Tricine/SDS sample buffer (Novex, Carlsbad, CA) was added to each reaction, and samples were boiled and subjected to electrophoresis on 10–20% gradient tricine

PAAG (Novex). Gels were dried and exposed to film with an intensifying screen at -70°C .

Data in all figures are shown as results of representative experiments. All experiments were repeated at least three times.

Results

Effect of Selenium Compounds on Prostate Carcinoma Cell Growth and Apoptosis. Clark *et al.* (3) found that the average plasma selenium level in participants treated with a supranutritional dose of selenium in prevention trial was around 2.5 μM . To establish an effective dose of selenium compounds for NF- κB *in vitro* studies, we first tested the effects of selenite and MSeA in concentration ranging from 1 to 5 μM on cell growth in the DU145 and JCA1 prostate cell lines. Both selenium compounds inhibited cell accumulation within 24 h of treatment in a dose-dependent manner (Fig. 1). Selenite appeared to be more effective than MSeA in both cell lines; it inhibited cell accumulation at all tested concentrations (1–5 μM). MSeA substantially inhibited cell accumulation only at 5 μM .

Apoptotic effects of selenite and MSeA at 5 μM have been studied in DU145 cells using as end points PARP cleavage and DNA fragmentation. Caspase-mediated cleavage of PARP inactivates this enzyme and inhibits its ability to respond to DNA strand breaks for repair. PARP cleavage is now recognized as one of the most sensitive markers of caspase-mediated apoptosis. As shown in Fig. 2A, at 24 h of exposure both compounds induced PARP cleavage. Consistent with our early findings (12), MSeA induced PARP cleavage more effectively than sodium selenite. As shown in Fig. 2B, at 24 h of exposure both compounds strongly and to the same extent induced nucleosomal fragmentation typical for apoptotic cell death.

Selenium Compounds Inhibited NF- κB DNA Binding Activity. To determine whether inhibition of prostate cell growth and induction of apoptosis correlated with selenium effect on NF- κB function, we studied the effect of selenite and MSeA on NF- κB DNA binding in DU145 and JCA1 cell lines, both of which we have characterized previously with regard to the basal and inducible NF- κB activity (26). On the basis of the data presented above, we have chosen the 5 μM dose for both selenium compounds to study their effects on NF- κB activation in prostate cells.

Because of the greater growth inhibitory response of JCA1 cells to selenium treatment (Fig. 1), we examined this cell line first for the impact of 30-min selenium preexposure on the TNF- α -induced NF- κB DNA binding activity by EMSA. Consistent with our previous findings, TNF- α strongly induced NF- κB DNA-binding in JCA1 cells (Fig. 3). MSeA-exposed cells showed considerably inhibited κB -binding at 3 h of TNF- α stimulation, but this effect was abolished by 6 h (Fig. 3). On the other hand, selenite-exposed cells had nearly full κB -binding activity by 3 h of stimulation but displayed much less binding by 6 h, indicating a more delayed action than that of MSeA. Analysis of NF- κB binding complexes by EMSA revealed that the major NF- κB complex in JCA1 cells is represented by the p50/p65 heterodimer (Fig. 3C).

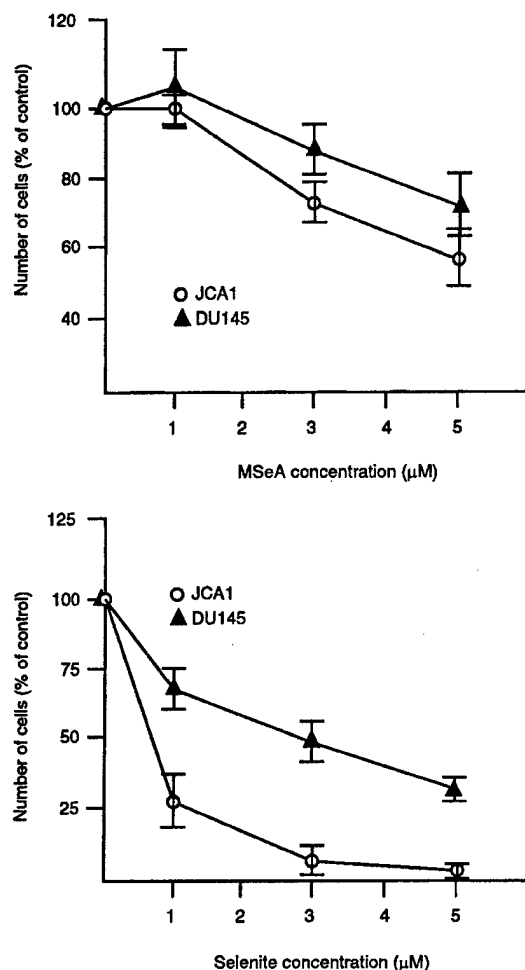


Fig. 1. Effect of selenium compounds on cell number accumulation in prostate cell cultures. DU145 and JCA1 cells were seeded in 12-well plates. At 50% confluence, cells were treated with MSeA or selenite at the indicated concentrations without medium change. The average number of cells in 3 wells/treatment was determined 24 h after the beginning of treatment. Data are shown as a percentage of control (means; bars, SD) for one representative experiment.

Similar patterns of differential impacts of selenite and MSeA on TNF- α -induced NF- κ B DNA binding activity were observed in DU145 cells (Fig. 4A). The kinetics of the inhibitory action and recovery in DU145 cells appeared to be faster than in JCA1 cells. Specifically, MSeA-exposed DU145 cells showed almost a complete block of NF- κ B binding at 0.5 h of TNF- α stimulation but almost fully recovered the binding activity by 3 h of stimulation. On the other hand, selenite-exposed DU145 cells showed considerably less binding by 0.5 h of TNF- α stimulation, and this effect persisted for 3 h. Selenite and MSeA preexposure also inhibited NF- κ B DNA binding induced by LPS (Fig. 4B). The major inducible NF- κ B complex in DU145 cells consisted of p50 and p65 proteins (Fig. 4C).

Taken together, the data support an inhibitory effect of selenium preexposure on the inducible NF- κ B DNA binding activity irrespective of the nature of stimulation. Furthermore,

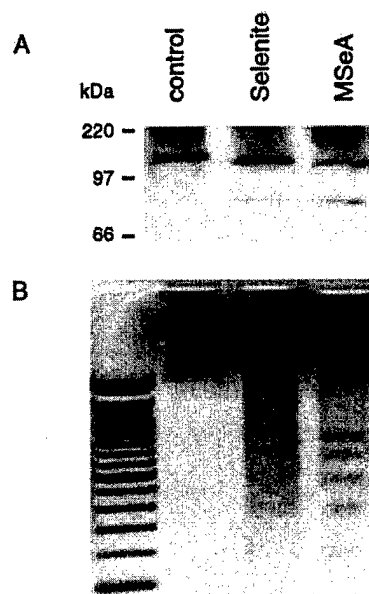


Fig. 2. Effect of selenium compounds on apoptosis in DU145 prostate cells. A, effect of selenium compounds on PARP cleavage. DU145 cells were treated with sodium selenite (5 μ M) and MSeA (5 μ M) for 24 h. PARP cleavage was defined by Western blotting with antibody, which detects both full-length PARP (116 kDa) and PARP cleavage product (85 kDa). Adherent cells and detached floaters were combined for whole-cell lysate preparations. B, effect of selenium compounds on DNA fragmentation. Agarose gel (1.5%) electrophoretic detection of nucleosomal DNA fragmentation in DU145 cells at 24 h of exposure with sodium selenite (5 μ M) and MSeA (5 μ M). The leftmost lane was loaded with DNA molecular weight marker. DNA was extracted from adherent cells combined with detached floaters. The inverted image was used to better show fragmented DNA.

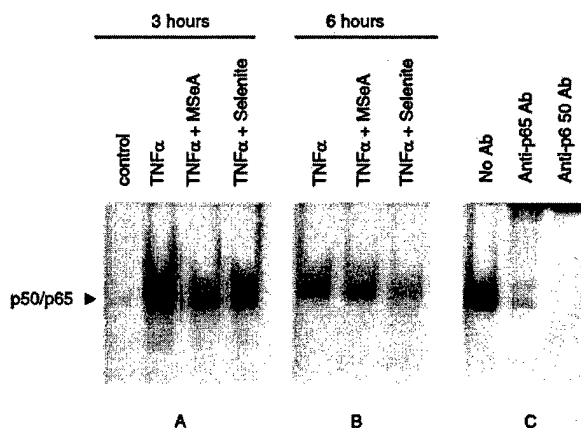


Fig. 3. Effect of selenium compounds on induced κ B binding in JCA1 prostate cells. A and B, EMSA analysis of κ B binding. Cells were pre-treated for 30 min with MSeA (5 μ M) or sodium selenite (5 μ M) without medium change. Then TNF- α (3.5 ng/ml) was added to the medium, and incubation was continued. Cells were harvested at the indicated time points, and nuclear and cytosol extracts were prepared. EMSA was performed by incubating nuclear proteins with a labeled κ B-oligonucleotide. C, identification of nuclear κ B binding complexes was analyzed by EMSA. Nuclear proteins from JCA1 cells were incubated with a labeled κ B nucleotide and antibodies against p50 and p65 proteins, and DNA binding was analyzed.

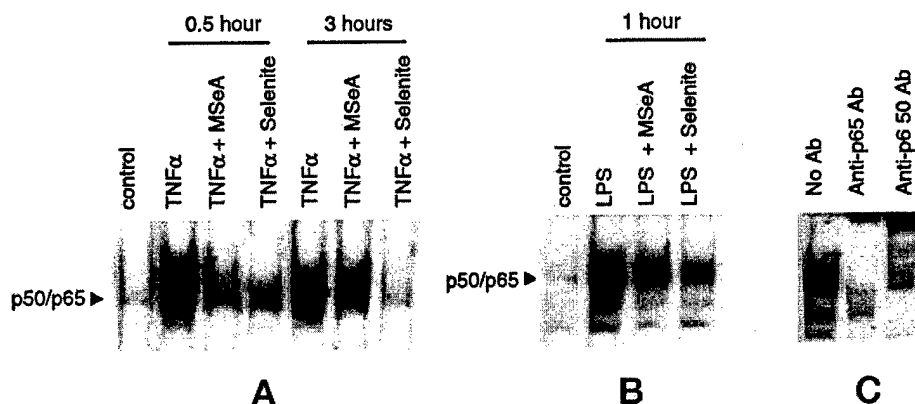


Fig. 4. Effect of selenium compounds on induced κ B binding in DU145 prostate cells. **A** and **B**, EMSA analysis of κ B binding. DU145 cells were pretreated for 30 min with MSeA (5 μ M) or sodium selenite (5 μ M). Then TNF- α (7.5 ng/ml) or LPS (1.5 μ g/ml) were added to the medium, and incubation was continued. Cells were harvested at the indicated time points, and nuclear and cytosol extracts were prepared. EMSA was performed by incubating nuclear proteins with a labeled κ B-oligonucleotide. **C**, identification of nuclear κ B binding complexes by EMSA. Nuclear proteins from DU145 cells were incubated with a labeled κ B nucleotide and antibodies against p50 and p65 proteins, and DNA binding was analyzed.

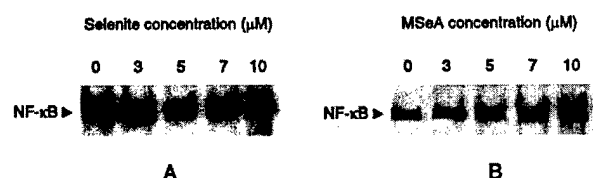


Fig. 5. Selenium compounds did not inhibit κ B DNA binding in cell-free protein extracts. Nuclear proteins (10 μ g/lane) from TNF- α -treated DU145 cells were incubated with a labeled κ B-oligonucleotide for 45 min and used for EMSA. MSeA and sodium selenite were added to the reaction mixture at the final concentrations 1–10 μ M for 15 min before the oligonucleotide.

persistent inhibition of NF- κ B activity appeared to correlate with the stronger growth-inhibitory activity of selenite than MSeA in both cell lines.

One of the previously proposed mechanisms of selenite effect on NF- κ B activity is a direct interaction between selenite and NF- κ B, which results in adduct formation with the thiol groups of NF- κ B proteins and subsequent alteration of NF- κ B properties including DNA binding (27). To test this hypothesis, we treated nuclear extracts from DU145 cells with increasing concentrations of selenite and MSeA (3–10 μ M) for 1 h, the time similar to the time of *in vivo* cell treatment with selenium in our experiments with DU145 cells. As shown in Fig. 5, no direct inactivating effect of selenium on κ B binding was observed by incubating nuclear extracts containing active NF- κ B complexes with both selenium compounds *in vitro*.

Selenium Inhibited NF- κ B-mediated Transcription Activity. To further investigate the selenium effect on NF- κ B function, we performed a transient transfection of prostate carcinoma JCA1 cells with an exogenous κ B-responsive gene, κ B-Luciferase reporter. Mindful of the apoptotic effects of prolonged selenium exposure, we have chosen 6-h exposure with both selenium compounds for these experiments. The results of transient transfection experiments presented in Fig. 6 correlated well with the EMSA results. Pretreatment

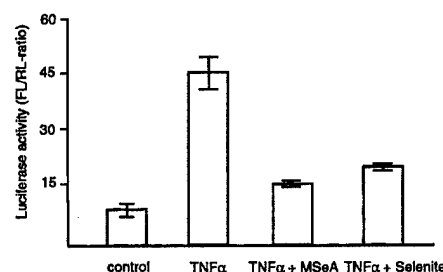


Fig. 6. Effect of selenium compounds on NF- κ B transcriptional activity in prostate cells. JCA1 prostate cells were cotransfected with 3 \times κ B-Luciferase reporter and pRL-null construct. Twenty-four h after transfection, cells were pretreated for 30 min with MSeA (5 μ M) or sodium selenite (5 μ M). Then TNF- α (7.5 ng/ml) was added to the medium, and incubation was continued for 6 h. Luciferase activity was measured by dual luciferase assay in cell lysates from three dishes/point. Data are shown as FL:RL ratio (means; bars, SD) for one representative experiment.

with both selenite and MSeA at 5 μ M significantly inhibited the luciferase activity induced by TNF- α in JCA1 cells.

Selenium Inhibited TNF- α and LPS-induced Activation of IKK. As we have already mentioned, the absence of a direct effect of both selenium compounds on NF- κ B DNA binding in a cell-free system (see Fig. 5) suggested that selenium affects some cellular steps in the NF- κ B activation pathway. One of the first key stages to NF- κ B activation is the phosphorylation of I κ B inhibitory proteins by IKK kinases. Thus, in our next experiment, we studied whether selenium compounds affected IKK activation and I κ B- α phosphorylation in DU145 cells.

As expected, TNF- α and LPS stimulation strongly increased IKK activity and increased the level of I κ B- α phosphorylation in DU145 cells (Fig. 7). Selenite and MSeA at a concentration of 5 μ M significantly inhibited IKK activation and subsequently I κ B- α phosphorylation induced by TNF- α and LPS. It is important to mention that the kinetics of IKK inhibition by two different selenium compounds correlated well with kinetics of inhibition of κ B DNA binding. The inhib-

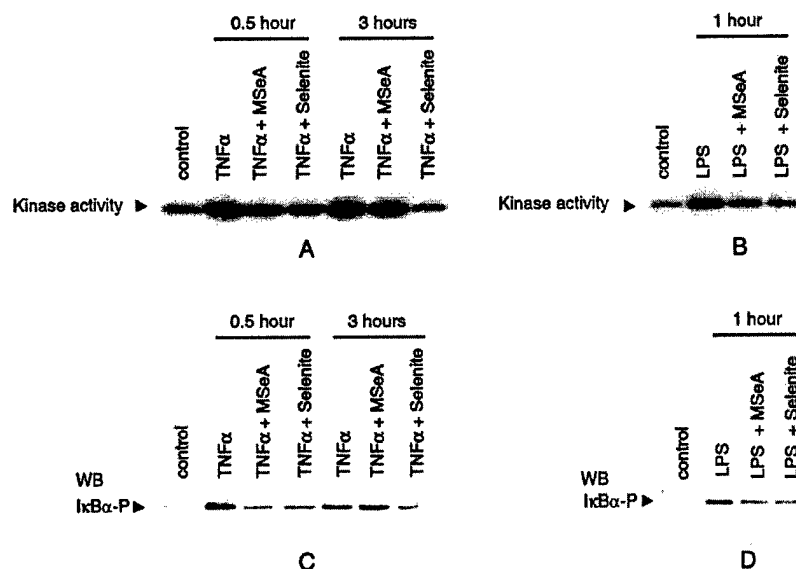


Fig. 7. Effect of selenium compounds on IKK activity and I κ B- α phosphorylation in prostate cells. A and B, IKK kinase activity. DU145 cells were pretreated for 30 min with MSeA (5 μ M) or sodium selenite (5 μ M) for 30 min. Then TNF- α (7.5 ng/ml) or LPS (1.5 μ g/ml) was added to the medium, and incubation was continued. Protein extracts from DU145 cells were prepared at the indicated time points, immunoprecipitated with a combination of IKK α and IKK β antisera, and used for *in vitro* kinase reaction. C and D, I κ B- α phosphorylation. In the same experiment, cells were harvested from the dishes at the indicated time points, and cytosol extracts were prepared. Western blots containing cytosol protein extracts (10 μ g/lane) were probed for expression of I κ B- α -P.

itory effect of selenite on IKK activity induced by TNF- α was more sustained than the effect of MSeA.

Selenium Decreased I κ B- α Degradation and p65 Nuclear Translocation. As we showed above, both selenium compounds blocked IKK activation and I κ B- α phosphorylation. This suggested that proteolytic degradation of I κ B- α should be also affected by selenium compounds. To determine whether selenium compounds indeed inhibited I κ B- α degradation, the cytoplasmic level of I κ B- α has been examined by Western blot analysis in DU145 cells pretreated with selenite and MSeA and treated with TNF- α . As we reported previously (26), TNF- α induced dramatic I κ B- α degradation within 10–30 min with complete I κ B- α resynthesis at later times of exposure in DU145 cells (Fig. 8). Both selenite and MSeA inhibited I κ B- α degradation during the first 30 min of cell exposure to TNF- α (Fig. 8). At later times, the effect of selenium compounds on I κ B- α level has not been revealed.

Whether selenium compounds affected TNF- α -induced nuclear translocation of the p65 subunit of NF- κ B was examined by Western blot analysis of nuclear protein extracts. As shown in Fig. 8, upon TNF- α treatment, p65 translocated to the nucleus in DU145 cells, and both selenite and MSeA inhibited this translocation. Consistent with the results on the repression of κ B DNA binding, the inhibitory effect of selenite on p65 translocation was more sustained than the effect of MSeA at 3 h.

NF- κ B Blockage Sensitized Prostate Carcinoma Cells to Apoptosis Induced by Selenium Compounds. We have extended our study further and studied the effect of NF- κ B blockage on the sensitivity of prostate carcinoma cells to apoptosis induced by selenium compounds. We have chosen for these experiments an I κ B α mutant with substitution of serines 32 and 36 to alanines (32A36A) that, because of the lack of putative phosphorylation sites, is not phosphorylated by IKKs and as a result does not undergo degradation. We have shown previously that this I κ B α mutant was able to block NF- κ B activity in prostate cells by 90–95% (28).

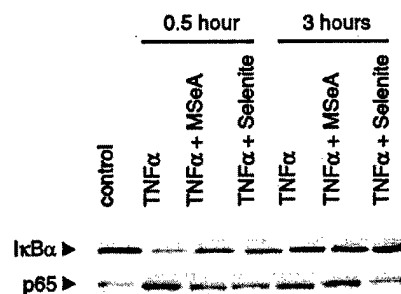


Fig. 8. Effect of selenium compounds on induced I κ B- α degradation and nuclear translocation of p65 in prostate cells. Cells were pretreated for 30 min with MSeA (5 μ M) or sodium selenite (5 μ M). Then TNF- α (7.5 ng/ml) was added to the medium, and incubation was continued. Cells were harvested at the indicated time points, and nuclear and cytosol extracts were prepared. Western blots containing cytosol protein extracts (10 μ g/lane) were probed for expression of I κ B α . Western blots containing nuclear protein extracts (10 μ g/lane) were probed for expression of p65.

We found that NF- κ B blockage in DU145 cells by infection with adenovirus construct encoding mutant human I κ B α protein (AdV-d.n.I κ B α) resulted in increased sensitivity of prostate carcinoma cells to both selenium compounds. Indeed, the morphological changes in DU145 cells in response to selenium compounds, such as profound cell retraction, rounding, and detachment described in our previous work (12), occurred much earlier in cell cultures infected with adenovirus expressing I κ B α mutant (data not shown).

As one could see in Fig. 9A, treatment of DU145 cells with sodium selenite or MSeA as well as cell infection with AdV-d.n.I κ B α resulted in PARP cleavage (Fig. 9, Lanes 3, 4, and 5). It is important that selenium treatment of DU145 cells with blocked NF- κ B resulted in more intensive PARP cleavage than in selenium-treated cells with intact, constitutively active NF- κ B (Fig. 9, compare Lanes 3 and 6 and Lanes 4 and 7). Moreover, because of a reduced expression of full-length PARP in MSeA- and selenite-treated cells with blocked NF-

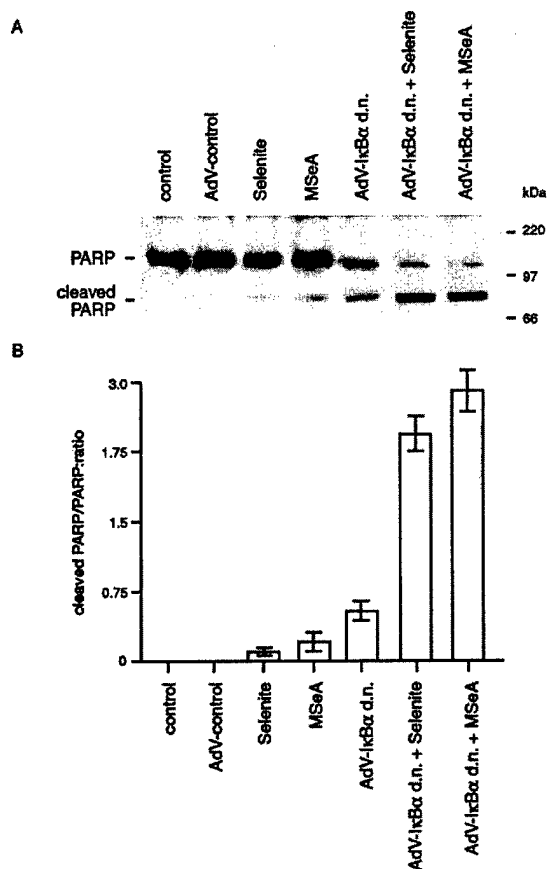


Fig. 9. Effect of $I\kappa B\alpha$ d.n. mutant on PARP cleavage in prostate cells. DU145 prostate cells were infected with adenovirus expressing GFP and $I\kappa B\alpha$ mutant lacking Ser-32 and Ser-36 (AdV-d.n. $I\kappa B\alpha$) or adenovirus expressing only GFP (AdV-control). Twenty-four h later, cell cultures were left untreated or treated with MSeA ($5 \mu M$) or with sodium selenite ($5 \mu M$) for 24 h. PARP cleavage was detected by Western blotting with antibody, which detects the full-length PARP (116 kDa) and PARP cleavage product (85 kDa). Adherent cells and detached floaters were combined for whole-cell lysate preparations. **A**, Western blot analysis of PARP cleavage. **B**, effect of $I\kappa B\alpha$ d.n. mutant and selenium compounds on cleaved PARP: full-length PARP ratio. Amounts of PARP and cleaved PARP presented on Western blots in **A** were quantified using One-Scan gel and blot analysis software, Scanalytics, Inc. (Fairfax, VA). Ratios of absorbances for bands corresponding to cleaved PARP and full-length PARP are plotted. Bars, SD.

κB , the ratio of cleaved PARP:full-length PARP was much strongly affected in those cells than in cells that underwent other treatments (Fig. 9B). Thus, both the amounts of cleaved PARP and the ratio cleaved PARP:full-length PARP were much higher in cells where NF- κB activity was inhibited by the expression of $I\kappa B\alpha$ d.n. mutant. These data clearly indicate that NF- κB blockage resulted in sensitization of prostate carcinoma cells to apoptosis induced by selenium compounds.

Discussion

Previous work has shown that selenite and MSeA represent two distinct pools of selenium metabolites (11, 29). Selenite and other selenium compounds that are metabolized to hy-

drogen selenide (H_2Se) induced DNA single-strand breaks and subsequent cell death by combination of acute lysis and apoptosis in different tumor cell cultures (12). Interestingly, selenite-induced apoptosis does not require caspase activation (12). Thus, less active PARP cleavage in prostate carcinoma cells treated with sodium selenite (Fig. 2A) probably reflects this caspase-independent type of apoptosis. MSeA and other immediate precursors of another major selenium metabolite, methylselenol, induced apoptosis mediated by caspase cascades (13) without genotoxicity and necrosis (11). In the work presented here, we have found that at an apoptotic dose, both sodium selenite and MSeA inhibited κB DNA-binding and NF- κB transcriptional activity induced by TNF- α or LPS in two prostate cancer cell lines. Specifically, the two selenium forms showed a differential kinetics of NF- κB inhibitory action; the effect of MSeA was more rapid and transitory, whereas selenite action was slower but persisted much longer. It is important to emphasize that NF- κB suppression by selenite, which exerted a more persistent effect on NF- κB function in comparison with the effect of MSeA, correlated well with the stronger inhibition of cell accumulation by this selenium compound in both cell lines (Fig. 1). Our results obtained in prostate cells are consistent with previous experimental findings that selenite could inhibit κB DNA binding in other mammalian cells (27, 30) and provided further biochemical insights on underlying mechanisms. In our experiments, we showed for the first time that selenium compounds inhibited function of NF- κB transcription factor through the blockage of IKK activity, the kinase complex that phosphorylates $I\kappa B$ inhibitors, and consequently, triggers $I\kappa B\alpha$ degradation. It is important to emphasize that NF- κB blockage by $I\kappa B\alpha$ d.n. mutant sensitized prostate carcinoma cells to apoptosis induced by selenium.

Although our research was focused on IKK as the important step necessary for NF- κB activation, there are other possible direct and indirect mechanisms of selenium effect on NF- κB function. It is known that selenium effects at lower doses are mediated by different selenium-proteins such as glutathione peroxidases, thioredoxin reductases, and others that have broad physiological functions including antioxidant protection and redox regulation of different protein activity (1, 31, 32). At higher doses, selenium can directly exert oxidation of nucleotides, proteins, and cofactors (1). One of the previously proposed mechanisms of the selenium effect on NF- κB activity is such a direct interaction between selenium and NF- κB , which results in adduct formation with the thiol groups of NF- κB proteins and subsequent alteration of NF- κB properties including DNA binding (27). The results of our experiments in cell-free system do not suggest that studied selenium compounds directly inhibit NF- κB DNA binding. Whether this discrepancy is caused by buffer conditions used in the test tube reactions or reflects a specific difference between cell types should be investigated.

How relevant are our findings in terms of cancer chemoprevention or therapeutic intervention by selenium?

(a) NF- κB factor activation has been associated with tumorigenesis. NF- κB is constitutively activated in human leukemias and lymphomas as well as in some solid tumors and in cell lines from human solid tumors including breast, ovar-

ian, colon, thyroid, pancreatic, and urinary bladder carcinomas, melanomas, and others (15, 33–38). It was shown that inhibition of NF- κ B activity in carcinoma cell lines could dramatically reduce cell growth and metastatic properties *in vivo* (39, 40). Recently, we found that NF- κ B is activated in some prostate carcinoma cell lines because of an aberrant activation of IKK, resulting in a high level of I κ B- α phosphorylation and turnover (28). These data suggest that constitutive activation of the NF- κ B signaling pathway could be an important step during tumor development in prostate. Therefore, selenium inhibition of NF- κ B activation during early stages of tumorigenesis, which are likely responsive windows for chemoprevention, could be one mechanism mediating prostate cancer prevention and treatment.

(b) NF- κ B plays a key role in cell protection against diverse apoptotic stimuli including chemo- and radiotherapeutic treatments through activation of the antiapoptotic gene program in cells (41). Antiapoptotic genes that are regulated by NF- κ B include genes encoding Bcl-2-like proteins (A1/Bfl1, Bcl-X_L, and Nr13), inhibitor of apoptosis proteins (H-IAP1, H-IAP2, and X-IAP1), and others (41). It became clear recently that NF- κ B is also an important regulator of cell proliferation. There is some evidence that NF- κ B proteins are implicated in cell cycle regulation through their effect on the cyclin-dependent kinase/cyclin-dependent kinase inhibitor system (42). The best explored link between NF- κ B activation and cell cycle progression involves cyclin D1, a cyclin that is expressed early in the cell cycle and is crucial to commitment to DNA synthesis (43). It was shown that promoters of cyclins D1 and D2 contain κ B binding sites and are important transcriptional targets of NF- κ B (43, 44). Therefore, inhibition of NF- κ B antiapoptotic and proproliferative activities could provide specific and causative links to inhibition of prostate tumor cell growth and survival by selenium compounds. In this regard, it is important to mention that *in vitro* the growth of prostate cancer cells was much more affected by selenium compounds than the growth of primary prostate cells (45).⁵

In conclusion, our study, as a proof of principle, established that an apoptotic dose of selenium exposure can inhibit NF- κ B activation through the inhibition of IKK activity and I κ B degradation. Because of the involvement of constitutive NF- κ B signaling in cancer cell survival and proliferation, our findings suggest that this signal pathway may constitute a molecular target for selenium to exert its anticancer activity. In this regard, it is noteworthy that some other potential cancer preventive natural compounds including green tea polyphenols, phytoestrogen genistein, vitamin E derivatives, silymarin, resveratrol, and curcumin have been also found to be inhibitors of NF- κ B and IKK in different cells (46, 47). It will be important to further identify the specific inhibitory mechanisms by each of these diverse agents to build a mechanistic basis for combinatorial application of multiple agents to synergistically inhibit NF- κ B signaling to achieve comprehensive prevention of prostate cancer.

⁵ I. V. Budunova and Y. J. Yao, unpublished data.

Acknowledgments

We thank Dr. O. Rokhlin (University of Iowa, Iowa City, IA) for providing JCA1 cells; Dr. N. Rice (NCI, Frederick, MD) for the generous gift of I κ B α N, IKK α , and IKK β antisera and technical support; and Dr. H. Ganther (University of Wisconsin, Madison, WI) for providing MSeA.

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(#1808) Prostate carcinoma cells secrete a soluble factor(s) responsible for NF- κ B activation. Alexander Gasparian, Alexander Yemelyanov, Ya Juan Yao, Dariusz Kowalczyk, and Irina Budunova. *AMC Cancer Research Centre, Denver, CO.*

We found recently that NF- κ B is constitutively activated in human androgen-independent prostate carcinoma cell lines DU145, PC3, and JCA1 but not in normal prostate epithelial cells and androgen-dependent PC cell line LNCaP. It was shown that prostate carcinoma cells produce and secrete some cytokines and growth factors that are potential NF- κ B activators. It is also known that some of these cytokines and growth factors are themselves regulated by NF- κ B at the transcriptional level. We hypothesized that increased NF- κ B activity in androgen-independent PC cells may be mediated by such factor(s) through an autocrine/paracrine loop. To test this hypothesis we incubated LNCaP cells with the conditioned medium of PC3 cells. Conditioned medium taken from PC3 cells induced I κ B α phosphorylation as well as κ B-binding in LNCaP cells. It also increased the expression of endogenous κ B-responsive genes such as I κ B- α . The conditioned medium of DU145 and JCA1 cells did not affect NF- κ B activity in LNCaP cells. Boiling and multiple freezing/thawing of PC3 conditioned media eliminated the effect of the medium on κ B binding. This suggests that the secreted NF- κ B inducer is a proteinaceous factor. The nature of the factor/factors that induce NF- κ B is under study. Supported by DOD Prostate Cancer Research Program DAMD17-01-1-0015.



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#2731 Role of IKK ϵ in NF-kappaB activation in prostate carcinoma cells.
Ya Juan Yao, Dariusz M. Kowalczyk, and Irina V. Budunova. *AMC Cancer Research Center, Denver, CO.*

Activation of NF- κ B transcription factor by different inducers employs a common pathway. I κ B inhibitory proteins, which retain NF- κ B in the cytosol, are rapidly phosphorylated by I κ B kinases (IKKs), ubiquitinated and undergo degradation. It became clear that NF- κ B activation requires multiple distinct IKKs. IKK ϵ (previously termed IKKi) is the only inducible IKK whose expression level is regulated by cytokines and possibly by NF- κ B itself. This suggests that IKK ϵ is involved in a positive autocrine loop of NF- κ B activation. NF- κ B transcription factor plays an important role in tumor development and is often constitutively activated in different tumor cells including androgen-independent prostate carcinoma (PC) cell lines (Gasparian, Budunova et al., 2001). The goal of this project was to study the role of IKK ϵ in constitutive and inducible NF- κ B activation in PC cells. Northern blot analysis revealed that IKK ϵ is expressed in DU145 and PC3 cells, which have high constitutive levels of NF- κ B activity, but not in primary normal prostate epithelial cells and in PC cell lines LNCaP and MDA PCa 2b which have low levels of NF- κ B activity. IKK ϵ transcription can be induced in PC cells by TNF- α treatment. The blockage of IKK ϵ function by stable transfection of PC3 cells with IKK ϵ dominant negative (d.n.) mutant K38A (Peters et al., 2000) resulted in the decrease of constitutive expression of endogenous κ B-responsive genes I κ B- α and IL6. The IKK ϵ d.n. mutant also significantly affected NF- κ B activation by selective NF- κ B inducers such as IL-1 and TPA. On the other hand, IKK ϵ d.n. mutant did not affect activation of NF- κ B-binding by TNF- α even though the degradation of I κ B- α in IKK ϵ d.n.-transfected cells was significantly blocked in comparison to parental and empty vector-transfected PC3 cells. These data indicate that IKK ϵ is necessary for NF- κ B induction by selected NF- κ B stimuli and possibly plays a significant role in constitutive activation of NF- κ B factor in androgen-independent PC cells. Supported by DOD Prostate Cancer Research Program DAMD17-01-1-0015.